Journal of Current Hematology & Oncology Research

Original Article

DOI: 10.51271/JCHOR-0038

The investigation of janus kinase 2 and calreticulin mutations in patients with essential thrombocytosis

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Cite this article: Doğan S, Yağcı AM. The investigation of janus kinase 2 and calreticulin mutations in patients with essential thrombocytosis. *J Curr Hematol Oncol Res.* 2024;2(3):54-58.

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Received: 12/02/2024 ◆ **Accepted**: 04/03/2024 ◆ **Published**: 01/08/2024

ABSTRACT

Aims: The aims of this study were to investigate the frequency of Janus kinase 2 (JAK2) and calreticulin (CALR) mutations in patients with essential thrombocytosis (ET) and primary myelofibrosis (PMF) and to compare the data of each group with JAK2 and CALR mutations (+) and (-).

Methods: The research group consisted of 80 patients with chronic myeloproliferative disease (CMPD) followed in Gazi University Faculty of Medicine, Department of Hematology.

Results: Of the patients included in the study, 66.2% had ET and 33.8% had PMF. JAK2 mutation (+) was detected in 60% and CALR mutation (+) was found in 22.5% of the patients. JAK2 mutation (+) was detected in 60.4% of patients with ET and 59.3% of patients with PMF. In JAK2 (-) patients, CALR was detected as (+) in 11 patients (52.4%) with ET and 7 patients (63.6%) with PMF. CALR (+) mutation rate was higher in female patients (n=15;83.3%) than males (3;16.7%)(p=0.022).

Conclusion: Studies in the literature have shown that the incidence of CALR mutations in patients with CMPD is between 28% and 80% and that the mutation is mostly seen in patients with ET. As a result of our study, it was concluded that CALR mutations (+) were similar to those in the literature and were more common in women and ET patients.

Keywords: Essential thrombocytosis, primary myelofibrosis, JAK2, calreticulin

INTRODUCTION

Chronic myeloproliferative diseases (CMPDs) were first described by William Damashek in 1951 as "abnormal increase in cell production as a result of genetic mutations in multipotent stem cells and abnormal growth of mature cell production in peripheral blood".[1,2] CMPDs are clonal diseases characterized by uncontrolled proliferation of one or more myeloerythroid cells in the bone marrow, with anomalies of hemostasis and thrombosis due to an increased number of mature and immature cells in peripheral blood and can progress to acute leukemia. There are four diseases in the CMPDs group: chronic myeloid leukemia, polycythemia vera, primary myelofibrosis (PMF) and essential thrombocythemia (ET).[3]

PMF is also known as myelofibrosis with myeloid metaplasia, angiogenic myeloid metaplasia and chronic idiopathic myelofibrosis.[4,5] PMF is a myeloproliferative neoplasm characterized by increased clonal neoplastic cells in the megakaryocytic sequence, fibrosis in the bone marrow and non-bone marrow hematopoiesis.[6] Pathogenesis includes megakaryocyte-dominant clonal proliferation, reactive bone marrow stromal changes, and extramedullary hematopoiesis. Approximately half of the patients have the Janus Kinase 2 (JAK2)V617F mutation.[7,8]

After the discovery of the (JAK2) mutation, the CMPD classification and diagnostic criteria changed, and the treatment algorithms were reshaped. In the criteria that the World Health Organization changed in 2008, presence of the JAK2 V617F mutation in the diagnosis of polycythemia vera, ET and PMF were included in the diagnostic criteria. The relationship between the JAK2 mutation and the severity of the disease has been demonstrated.[9]

The mutation in calreticulin (CALR) has been discovered in recent years. CALR is an endoplasmic reticulum protein with chaperone activity that plays a role in calcium proliferation and differentiation in cell proliferation. CALR dysfunction has been associated with various cancers. Finally, CALR mutations have been associated with JAK 2 (-) CMPD.

The primary aim of our study was to investigate the frequency of JAK2 mutation and CALR mutation in ET and PMF patients. And the secondary aim was also to investigate

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the relationship between JAK2 or CALR mutation (+) and (-) patients with thromboembolic events, bleeding, acute leukemia, myelofibrosis, age, sex and laboratory findings.

METHODS

The study included 80 patients with ET and PMF who were treated at Gazi University Medical Faculty Hospital Hematology Clinic. An approved consent form was obtained from all patients in writing and the study was performed in accordance with the principles of the Helsinki Declaration.

Apart from the CALR mutation analysis, all of the parameters used in the research were routinely required. JAK2 mutation analysis, age, sex, spleen and liver size, complete blood count, biochemistry, bone marrow examination, the presence of structural symptoms, additional diseases, bleeding, thrombosis, leukemic transformation, and itching were used in the parameterized study. Peripheral blood samples of the patients were taken. DNA was isolated from patient samples and the DNA obtained from the CALR mutation was examined.

Calreticulin Analysis

DNA was isolated from peripheral blood samples using the Qiagen-QIAmp DNA Blood Mini Kit(50) (Reference No. 51104) in accordance with the kit instructions.Using a NanoDrop device, the quality of the DNA samples was checked to ensure that approximately 100 ng of DNA was obtained and the Real-Time PCR mix was prepared. Qiagen CALR RGQ PCR Kit (24) was used for Real-Time PCR. For each sample, 3 controls were used and 7 mutations, 2 of which were major, were scanned. The analyses were carried out according to the kit instructions and the results were given qualitatively. The study was carried out with the permission of Ethical Committe of Faculty of Gazi University Clinical Research Ethics (Date:11.01.2016, Decision No: 13).

Data Analysis

The data were analyzed with SPSS 22.0 (Statistical Package for Social Science) program. The normal distribution of the variables was evaluated with the Kolmogorov Smirnov (KS) test and the variables with normal distribution were compared with T-test. Variables that did not meet the normal distribution were compared with Kruskal Wallis H (KW-H) test. Chi-square test was used for the analysis of categorical variables. Data are shown as averages, standard deviations, standard errors of mean, minimum and maximum values in percentages. Results were compared with a 95% confidence level and p <0.05 was considered statistically significant.

RESULTS

Demographic Features

The distribution of demographic characteristics according to the diagnoses of the cases included in the study was shown in Table 1. As seen in the table, 66.2% of 80 cases were diagnosed as ET and 33.8% were diagnosed with PMF.

Table 1. Distribution of gender and age averages with essential thrombocytosis (ET) and primary. JAK2 mutation distribution

Demographic Features		Number of People(n) (%) ET-PMF	Diagnosis Total and Rate		
Gender	Female	n	35	16	51
		%	66	59.3	63.8
	Male	n	18	11	29
		%	34	40.7	36.2
Total		n	53	27	80
		%	66.2	33.8	100
Age (year)	Mean±SD		55.8±15.1	63.9±11.8	58.6±14.5

The mean age at the time of diagnosis was 58.6 ± 14.5 years. There was no statistically significant difference between the sexes of the cases and ET and PMF diagnoses (p> 0.05). However, the mean age of patients with PMF was higher than patients with ET and this difference was statistically significant (p<0.05).

JAK2 mutation (+) was found in 60% (n=48) of 80 patients included in the study and JAK2 mutation (-) in 40% (n=32). JAK2 mutation (+) was detected in 60.4% (n=32) of the cases with ET. JAK2 mutation (+) was found in 59.3% (n=16) with PMF.

The mean age of the patients with JAK2 mutation (+) was 59.7 ±14 years and was higher than the mean age of the patients with JAK2 mutation (-) (56.9±15.2 years). However, no significant difference was found in gender and age for cases with and without JAK2 mutation (p=0.492 and p=0.777, respectively).

The Relationship Between JAK2 Mutation and Laboratory/ Clinical Findings

There was no statistically significant difference (p> 0.05) between platelet, white blood cell, lactate dehydrogenase (LDH) and survival time in ET patients with JAK2 mutation (+) and (-); however, ET patients with JAK2 mutation (+) had higher hemoglobin and hematocrit values compared to mutation (-) cases and this difference was statistically significant (p=0.030 and p=0.008, respectively).

In patients with PMF, no statistically significant difference was found between the laboratory values of JAK2 mutation (+) and (-) patients (p>0.05).

There was no statistically significant difference between patients with JAK2 mutation (+) and (-) in patients with ET, splenomegaly, hepatomegaly, bleeding, thrombosis, structural symptoms, treatment, itching and transformation to leukemia (p> 0.05). The presence of fibrosis in the bone marrow was higher in the JAK2 mutation (+) cases (59.4%); mutation was less observed in (-) cases (28.6%). However, this difference was not statistically significant (p=0.084).

There was no statistically significant difference between the patients with JAK2 mutation (+) and (-) in patients with PMF, bone marrow fibrosis, splenomegaly, hepatomegaly, bleeding,

thrombosis, structural symptoms, treatment, itching, disease return to leukemia and additional diseases (p>0.05).

Calreticulin Mutation and Its Distribution

Of the 32 patients with JAK2 mutation (-), 18(56.2%) had a CALR mutation (+) and 14(43.8%) had a CALR mutation (-). The rate of patients with CALR mutation (+) was 22.5% of all subjects (n=80) and 17.5% of patients with CALR mutation (-). When a comparison was made according for sexes, it was observed that CALR (+) mutation rate was higher in female patients (n=15;83.3%) than males (3;16.7%) and this difference was statistically significant (p=0.022).

Laboratory and Clinical Findings in Patients With JAK2 Mutation (+), CALR Mutation (+) and Both Mutations (-)

There was no statistically significant difference between platelet, white blood, hemoglobin, LDH values and survival times of the patients with JAK2 mutation (+), CALR mutation (+) and JAK2 (-)CALR mutations (-) (p>0.05). However, there was a statistically significant difference between the groups with the hematocrit values of the CALR mutation (+) group lower than the other two groups (p=0.048).

Bone marrow fibrosis, splenomegaly, hepatomegaly, bleeding, thrombosis, structural symptoms, treatment, pruritus, transformation to leukemia, and the incidence of comorbidities was not statistically significant in patients with a JAK2 mutation (+), a CALR mutation (+) and a JAK2(-) CALR(-) mutation (p> 0.05). Frequency of CALR mutations were mentioned in Table-2.CALR mutation (+) was found in 11(52.38%) patients with JAK2 mutation (-), and CALR (-) in 10(47.61%) patients. 7 (63.63%) patients with PMF were CALR mutation (+) and 4 (36.36%) CALR (-) (Figure 1).

Table 2. Frequency of Calreticulin (CALR) mutation types.

Result (n,%)	In JAK2 (-) (n=32) Frequancy / Rate		Totally (n=80) Rate	CALR Mutation Positive (n=18)
CALR Mutation (-)	14	43.8	17.5	
CALR Mutation (+)	18	56.2	22.5	
Minor Mutation	4	12.5	5	22,2
Type 1 Mutation and Minor Mutation	8	25	10	44,4
Type 2 Mutation and Minor Mutation	2	6.2	2,5	11,1
Type 1 and Type 2 Mutation and Minor Mutation	1	3.1	1,3	5,6
Type 1 Mutation	2	6.2	2.5	11.1
Type 2 Mutation	1	3.1	1.3	5.6

CALR types and frequency data of ET and PMF patients mentioned above did not show a statistically significant difference (p>0.05) (Table 3).Table 4 shows the frequency of (-) and (+) CALR mutations diagnosed with 21 ET and 11 PMF patients with a (-) JAK2 mutation.

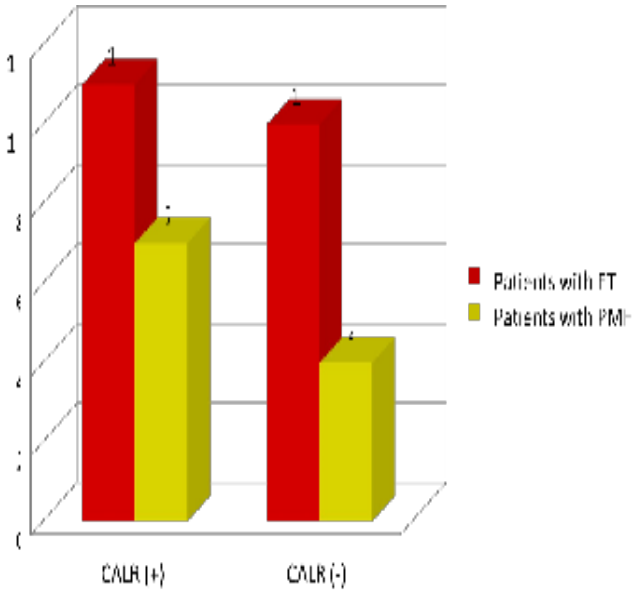

Figure. CALR numbers of ET and PMF with JAK2(-)

Table 3. Frequency of Calreticulin (CALR) mutation types in ET and PMF patients with JAK2 (-).

CALR (+) Numbers and Types	ET (n=21)		PMF (n=11)		
	n	%	n	%	p
CALR 1 Major	5	23.8	6	54.5	0.123
CALR 2 Major	3	14.28	1	9.1	1
CALR Clamp 1 Minor	5	23.8	4	36.4	0.681
CALR Clamp 2 Minor	5	23.8	3	27.3	1
CALR Clamp 3 Minor	2	9.5	-	-	0.534
CALR Clamp 4 Minorss	2	9.5	1	9.1	1
CALR Clamp 5 Minor	7	33.3	1	9.1	0.209

DISCUSSION

The presence of JAK2 mutation in ET and PMF patients is an important finding for diagnosis and has led to an increase in the knowledge of the genetic basis of these diseases.[10] The rate of JAK2 mutation (+) in ET and PMF patients is different in the literature. This rate was 23.2%-79.2% in ET patients; PMF patients ranged from 37% to 78%.[9,10-16] In two studies in Turkiye, JAK2 mutation in ET patients (+) was at a rate of 40% and 47.6%.[2,16] In our study, JAK2 mutation (+) was found in 60% of patients with ET and 59.3% of patients with PMF. Our findings are consistent with most of the information in the literature. The presence of JAK2 mutation (+) in ET and PMF patients was found to be associated with elevated hemoglobin levels, high leukocyte count and low platelet count in ET patients, except for high age of diagnosis.[17] Similarly, the JAK2 mutation (+) was found to increase the tendency to itch in polycythemia vera and PMF patients.[18] In our study, it was determined that most of ET and PMF patients with JAK2 (+) and (-) did not have pruritus, and it was found that there was no statistically significant difference between the groups according to pruritus. This finding differs from the literature findings.

Table 4. Frequency of Calreticulin (CALR) mutation types in ET and PMF patients with JAK2

Result	Number of People / Rate	Diagnosis		Total
		ET	MF	
Mutation (-)	n	10	4	14
	% (In Group)	71.4	28.6	100
	% (In ET and PMF Patients)	47.6	36.4	43.8
	% (In JAK2 Mutation (-) ones)	31.2	12.5	43.8
Type1 Mutation, Minor Mutation	n	4	0	4
	% (In Group)	100	0	100
	% (In ET and PMF Patients)	19.0	0	12.5
	% (In JAK2 Mutation (-) ones)	12.5	0	12.5
Type2 Mutation, Minor Mutation	n	4	4	8
	% (In Group)	50	50	100
	% (In ET and PMF Patients)	19	36.4	25
	% (In JAK2 Mutation (-) ones)	12.5	12.5	25
Type1 ve Type2	n	1	1	2
	% (In Group)	50	50	100
	% (In ET and PMF Patients)	4.8	9.1	6.2
	% (In JAK2 Mutation (-) ones)	3.1	3.1	6.2
Type1 Mutation	n	1	0	1
	% (In Group)	0	2	2
	% (In ET and PMF Patients)	0	100	100
	% (In JAK2 Mutation (-) ones)	0	18.2	6.2
Type2 Mutation	n	0	6.2	6.2
	% (In Group)	100	0	100
	% (In ET and PMF Patients)	4.8	0	3.1
	% (In JAK2 Mutation (-) ones)	3.1	0	3.1
Total	n	21	11	32
	% (In Group)	65.6	34.4	100
	% (In ET and PMF Patients)	100	100	100
Mutation, Minor Mutation	% (In JAK2 Mutation (-) ones)	65.6	34.4	100
	% (In Group)	100	0	100
	% (In ET and PMF Patients)	4.8	0.0	3.1
	% (In JAK2 Mutation (-) ones)	3.1	0	3.1

Studies have shown that there is a statistically significant relationship between JAK2 mutation in ET and PMF patients with high hemoglobin levels, increased leukocyte count, low platelet count at diagnosis, increased arterial/venous thrombosis risk and presence of splenomegaly.[19,20] Demir et al. found higher leukocytes, hemoglobin and hematocrit values and incidence of thrombosis in ET patients with JAK2 mutation, in their research which was conducted in Turkiye.[21] In our study, no significant difference was found in platelet, leukocyte, hemoglobin, LDH values and mean survival time of JAK2 mutation (+) and (-) patients. However, there was a significant difference between hemoglobin and hematocrit values and hemoglobin and hematocrit values of ET patients with JAK2 mutation (+), which were higher (p=0.030 and p = 0.008). This finding is consistent with the literature.

In ET patients, old age (≥60 years), leukocytosis (≥11.000/mm³) and thrombosis (arterial or venous) are risk factors for survival. Similarly, anemia and an excessively high platelet count (>1.500.000/mm³) were found to increase the risk of leukemic transformation.[22] Passamonti et al reported that 10.6% of 605 patients died during the study period, and the median survival was 22.3 years.[23] In our study, the majority of patients with JAK2 mutation (93.8%) had no transformation to leukemia, 2 patients (6.2%) transformed to AML; there were no significant differences in the number of JAK2 mutations (+) (95.8%), no transform to leukemia, and transform to AML in 1 patient (4.2%) and there was no statistically significant difference between the mutation (+) and (-) patients in return to leukemia.

In the literature, the CALR mutation (+) ratio in ET and PMF patients varies between 20% and 100%. In one study, 20% to 25% of CALR somatic mutations were detected in all exon studies with new generation sequencing in ET and PMF patients with JAK2 mutation (-), respectively.[24] In another study, the frequency of CALR and JAK2 in patients with PMF and the effects of this mutation on prognosis and clinical status were investigated and CALR mutation (+) was detected in 22.7% of 617 patients.[25] In the study of Klampfl et al.,[16] 60% of 311 patients with CMPD CALR mutation (+) were JAK2 (-) for all patients with ET (100%). The CALR mutation (+) was determined for 72 patients with polycythemia vera.[26] In the study of Nangalia et al.,[27] CALR mutation was detected in 84% of patients with ET and MF with JAK2 mutation (-). In another study conducted by Tefferi et al, 407 of 1027 patients with PMF had ET and 111 (28%) of these ET patients had a CALR mutation.

In our study; CALR mutation (+) was found in 18 (22.5%) of 80 patients. The number of female patients with CALR mutation (+) was found to be higher than the number of male patients and it was found that this difference was statistically significant. The CALR mutation (+) or (-) did not differ according to age. CALR mutation (+) was found in 52.38% of patients with ET with JAK2 (-). CALR (+) was found in 63.63% of patients with PMF. There was no statistically significant difference between the groups in terms of (+) and (-) CALR frequency.

CALRdel52 (type 1 mutation) and CALRins5 (type 2 mutations) are the most common mutations in ET and PMF patients and type 1 mutation has been reported to be more frequent in PMF than in type 2.[28] In our study, it was detected that among patients with PMF; there were CALR mutation (-) in 4 patients (36.4%), Type 1 and minor mutation in 4 (36.4%), Type 2 and minor mutation in 1 (9.1%) patients Type 1 mutation in 2 patients (18.2%).

There are some limitations in our study. Our most important limitation is that it is a retrospective study. Our other limitation is the lack of generalizability of the findings due to the single center experience.

CONCLUSION

In conclusion, the findings of the CALR mutation are similar to the findings in patients with JAK2 mutation, as in the literature, and it is thought that the presence of CALR mutation in patients with JAK2 mutation (-) will support the diagnosis. This study is the first study in our country to investigate the presence of CALR mutation in ET and PMF. Therefore, we believe that this study will serve as an example for other studies in terms of using ET and PMF patients for diagnostic purposes in the investigation of JAK2 and CALR mutation.

ETHICAL DECLARATIONS

Ethics Committee Approval

The study was carried out with the permission of Ethical Committe of Faculty of Gazi University Clinical Research Ethics (Date:11.01.2016, Decision No: 13).

Informed Consent

All patients signed and free and informed consent form.

Referee Evaluation Process

Externally peer-reviewed.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

Financial Disclosure

The authors declared that this study has received no financial support.

Author Contributions

All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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